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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/698,323	10/27/00	ISNER J	47624-DIV (1

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EXAMINER	
NGUYEN,Q	
ART UNIT	**PAPER NUMBER**
1632	5

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No.	Applicant(s)	
	09/698,323	ISNER ET AL.	
	Examiner	Art Unit	
	Quang Nguyen	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-31,48 and 49* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-31,48 and 49* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☒ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15) ☒ Notice of References Cited (PTO-892)
16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
18) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other:

DETAILED ACTION

The preliminary amendment B, filed on 27 October 2000, in Paper No. 3 has been entered. Claims 1-31, 48 and 49 are pending in the present application.

Oath/Declaration

The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

> The oath or declaration is defective because: the date of the declaration executed by Applicants is omitted.

Claim Objections

Claim 21 is objected to because of the following informalities: presumably the term "agent" is totally missing in the claim. Appropriate correction is required.

Claim 23 is objected to because of the following informalities: a space between the term "oxidesynthase" is needed. Appropriate correction is required.

Specification

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code (See specification on page 18, line 9). Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP § 608.01.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-31 and 48-49 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods known in the art at the time of effective filing of the present application for formation of new blood vessels in a mammal having chronic or acute ischemia; the same wherein the vascularization modulating agent is GM-CSF, M-CSF, b-FGF, SCF, SDF-1, G-CSF, HGF, angiopoietin-1, angiopoietin-2 or FLT-3 ligand; the same wherein GM-CSF protein is administered to the mammal in an effective amount to increase the concentration and differentiation of endothelial progenitor cells (EPC) in the mammal; the same further comprising the co-administration with at least one angiogenic protein; a method for enhancing EPC mobilization in a mammal wherein the method comprises administering an effective amount of at least one hematopoietic factor sufficient to enhance the EPC mobilization in the mammal and the same further comprising the co-administration of an effective amount of one or more of GM-CSF or at least one angiogenic protein, does not reasonably provide enablement for other embodiments of the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

Claims 1-24 are drawn a method for inducing formation of new blood vessels in a mammal, preferably one that is suspected of having or will have ischemic tissue, wherein the method comprises administering to the mammal an effective amount of a **vascularization modulating agent** sufficient to form new blood vessels in the mammal; the same method wherein the vascularization modulating agent is GM-CSF, M-CSF, b-FGF, SCF, SDF-1, G-CSF, HGF, angiopoietin-1, angiopoietin-2, FLT-3 ligand, or **an effective fragment thereof**; the same method wherein the vascularization modulating agent is GM-CSF, and amount of the GM-CSF administered to the mammal is sufficient to increase the concentration and the differentiation of endothelial progenitor cells (EPC) in the mammal; the same method wherein the amount of said agent is sufficient to increase endothelial progenitor cells (EPC) differentiation following tissue ischemia or sufficient to increase bone marrow derived EPC incorporation into foci of neoangiogenesis. Claims 21-24 are directed to the same method wherein the agent is co-administered with at least one angiogenic protein, preferably those recited in the claims.

Claims 25-31 are drawn to a method for **preventing or reducing the severity of blood vessel damage** in a mammal, wherein the method comprises administering to the mammal an effective amount of granulocyte macrophage-colony stimulating factor (GM-CSF), and exposing the mammal to conditions conducive to damaging the blood vessels, the amount of GM-CSF being sufficient to prevent or reduce the severity of the blood vessel damage in the mammal; the same method wherein said conditions are an invasive manipulation or ischemia, preferably under specific recited conditions.

Claims 48 and 49 are directed to a method for enhancing endothelial progenitor cell (EPC) mobilization in a mammal, wherein the method comprises administering an effective amount of at least one hematopoietic factor sufficient to enhance the EPC mobilization in the mammal; the same further comprising co-administering to the mammal an effective amount of one or more of GM-CSF, at least one angiogenic protein, or **an effective fragment thereof**.

The specification teaches that intraperitoneal injection of recombinant mouse GM-CSF into mice for 7 days resulted in an increased circulating EPC at day 0 prior to creating a stimulus for neovascularization in treated mice (for this instance the creation of the cornea micropocket and insertion of VEGF pellet). The GM-CSF pretreatment enhanced the neovascularization process observed for treated animals versus untreated animals. Additionally, in a rabbit model of induced hindlimb ischemia, the pretreatment of recombinant human GM-CSF via subcutaneous daily injection for 7 days prior to the development of hindlimb ischemia also resulted in an enhanced EPC mobilization prior to and after the operation, as well as extensive neovascularization and

improved ischemic/normal hindlimb blood pressure ratio following the onset of ischemia, relative to control animals. Utilizing a mouse bone marrow transplantation model, the instant specification also teaches that the EPCs contributing to enhanced corneal neovascularization were specifically mobilized from the bone marrow in response to ischemia and GM-CSF. The specification also discloses the isolation of EPC-enriched cell fractions from mice as Sca-1 antigen positive (Sca-1+) cells, and from rabbits as a cell population depleted of T-lymphocytes, B-lymphocytes and monocytes (TBM^-). These cell populations have been shown to differentiate *in vitro*, and the differentiated cells showed evidence of EC lineage by reaction with BS-1 lectin and uptake of acetylated LDL. Furthermore, upon intravenous administering DiI-labeled Sca-1+ or autologous DiI-labeled TBM^- cells into mouse and rabbit hindlimb ischemia models, respectively, the labeled EPC-derived cells were shown to differentiate *in situ* into ECs as indicated by the co-staining for CD 31 (PECAM) and that the labeled cells were incorporated into colonies, sprouts and capillaries. The specification further teaches that EPC-enriched populations were increased in circulating blood following the onset of induced ischemia in the mouse and rabbit models, and the ischemia-induced EPC mobilization was demonstrated by enhanced ocular neovascularization (monitored by biomicrocopic and fluorescent microscopic examinations) after cornea micropocket surgery in mice with hindlimb ischemia.

The above evidence has been noted and considered. However, the instant specification is not enabled for the instant broadly claimed invention for the following reasons.

When read in light of the specification, a vascularization modulating agent encompasses a vascularization modulating protein or polypeptide, a fragment thereof or a DNA encoding the same (See specification, page 4 line 28 continues to line 4 on page 5). As such, for claims reciting a vascularization modulating agent, an embodiment of the claims falls within the realm of gene therapy. At the effective filing date of the present application (03/09/1998), gene therapy was highly unpredictable and immature. Even in a recent meeting review article on gene therapy and translational cancer research, Dang et al. (Clin. Cancer Res. 5:471-474, 1999) stated that "This workshop reviewed some recent advances in gene delivery, gene expression, immune manipulation, and the development of molecular targets and stressed that all of these fields will need further advancement **to make gene therapy a reality**" (page 471, column 1, last sentence of first paragraph). Dang et al. particularly noted that "Although significant progress has been achieved in our understanding of the limitations of gene therapy by suboptimal vectors, host immunological responses to the vectors, and the lack of long term stable expression, **the major challenge that limits clinical translation remains in achieving efficient gene delivery to target tissues**" (page 474, column 2, last paragraph). The instant claims encompass any and all routes of administering to the mammal an effective amount of a vascularization modulating agent. However, the specification fails to provide any guidance regarding how to administer any vascularization modulating agent in the form of a DNA to target cells or tissues in a mammal by any modes of delivery, such that an effective induction of new blood vessels in the mammal would occur. Nor does the instant specification provide specific

teachings regarding to which specific cell or tissue that a vascularization modulating agent in the form of a DNA should be targeted to for its expression for the induction of an effective mobilization of EPC in the mammal. Apart from the exemplification demonstrating that intraperioneal and subcutaneous injections of recombinant GM-CSF in mice and rabbits, respectively, to mobilize circulating EPC in the mammals, the instant specification fails to provide any guidance regarding to the use of a DNA encoding the same or other vascularization modulating agents for the same purpose. Since the prior arts at the effective filing date of the present application do not provide such guidance, it is incumbent upon the instant specification to do so, particularly in view of the unpredictable nature of the gene therapy art. Moreover, at the effective filing date of the present invention, *in vivo* vector targeting continues to be unpredictable and highly inefficient. This is supported by numerous teachings in the art. For examples, Miller & Vile (FASEB 9:190-199, 1995) reviewed the types of vectors available for *in vivo* gene therapy, and concluded that ""for the long-term success as well as the widespread applicability of human gene therapy, there will have to be advances ...: Targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated into components of safe and highly efficient delivery systems" (page 198, column 1). Deonarain (Exp. Opin. Ther. Patents 8:53-69, 1998) indicated that one of the biggest problems hampering successful gene therapy is the "ability to target a gene to a significant population of cells and express it at adequate levels for a long enough period of time" (page 53, first paragraph). Deonarain also reviewed new techniques under experimentation in the art which show promise, but is

currently even less efficient than viral gene delivery (see page 65, first paragraph under Conclusion section). Verma & Somia (Nature 389:239-242,1997) reviewed various vectors known in the art for use in gene therapy and the problems which are associated with each and clearly indicated that the resolution to vector targeting had not been achieved in the art (see the entire article). Verma & Somia also discussed the role of the immune system in inhibiting the efficient targeting of viral vectors such that efficient expression is not achieved (see page 239, and second and third columns of page 242). Verma & Somia further indicated that appropriate enhancer-promoter sequences can improve expression, but that the "search for such combinations is a case of trial and error for a given cell type" (page 240, sentence bridging columns 2 and 3). The specification fails to provide sufficient guidance for a skilled artisan how to overcome the unpredictability of *in vivo* vector targeting, such that an efficient transgene transfer and expression could be achieved in specific target cells or tissues to induce new blood vessels or for the effective mobilization of EPC in a mammal by any modes of delivery in the methods as claimed. Furthermore, the instant specification fails to provide any guidance regarding to the issue whether a stable *in vivo* transgene expression for a vector encoding any vascularization modulating agent **could be achieved to mobilize an effective level of EPC for the neovascularization process**. As noted above, the lack of a stable *in vivo* transgene expression and the lack of an optimal expression vector are some known factors limiting the effectiveness of gene therapy. Additionally, with respect to the breadth of the claims encompassing any and all vascularization modulating agents, factors such as the level of mRNA produced, the stability of the

protein produced, the protein's proper compartmentalization within the cell or its secretory fate differ dramatically based on which protein being produced, and therefore the desired outcome sought to achieve (Eck & Wilson, Gene-based therapy, page 81, column 2, last paragraph continues to the first paragraph of column 1 on page 82). Thus, the level of transgene expression, its duration and its *in vivo* effects are not always predictable, and these can not be overcome with routine experimentation. Accordingly, given the lack of guidance provided by the instant specification regarding to the issues raised above, it would have required undue experimentation without a predictable expectation of success for one skilled in the art to make and use the broadly claimed invention.

The instant claims encompass any effective fragment of a vascularization modulating agent and more preferably any effective fragment of the recited vascularization modulating agents such as GM-CSF, HGF, angiopoietin-1, angiopoietin-2, FLT-3 ligand and others. The specification is not enabled for such a broadly claimed invention. This is because there is a high degree of unpredictability associated with the use of the claimed embodiments, specifically the specification fails to teach which amino acids to be substituted, deleted or inserted, at which positions and in which combinations for a fragment of a vascularization protein or a DNA encoding the same such that the protein fragment or the encoded protein fragment still possess the angiogenic activity or enhancing the mobilization of endothelial progenitor cells. The unpredictability of the broadly claimed invention is further underscored by the absence of information concerning the stability and the proper folding for a functional fragment of

a vascularization modulating agent. In discussing peptide hormones, Rudinger has stated that "The significance of particular amino acids and sequences for different aspects of biological activity can not be predicted a priori but must be determined from case to case by painstaking experimental study (Page 6, first sentence of Conclusions *In* J.A. Parsons, ed. "Peptide hormones", University Park Press, 1976). Furthermore, the relationship between the sequence of a peptide and its tertiary structure associated for its activity is not well understood and is not predictable (Ngo et al., *In* Merz et al., ed. "The protein folding problem and tertiary structure prediction", Birkhauser, 1994). Additionally, the physiological art is recognized as unpredictable (MPEP 2164.03). As set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> That scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the are; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictagle factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved.

Therefore, in the absence of sufficient guidance provided by the instant specification regarding to the use of any fragment of a vascularization modulating fragment in the methods as claimed, it would again require undue experimentation without a predictable expectation of success for a skilled artisan to make and use the instant broadly claimed invention.

The breadth of the broad claims encompasses the induction of new blood vessel formation in any and all mammals, including those that **do not** suffer any acute or chronic ischemia. Apart from the exemplification shown by the present application

utilizing animal models of induced ischemia and the standard cornea (an avascular tissue) micropocket assay, the instant specification fails to provide any guidance demonstrating that a vascularization modulating agent could induce angiogenesis in any normal non-ischemic vascular tissue of a mammal as broadly claimed. Nor did the prior arts at the effective filing date of the present invention provide sufficient guidance in this regard. Safi et al. (J. Mol. Cell Cardiol. 29:2311-2325, 1997) have raised the question "Is ischemia required for the angiogenic factor to exercise its effect, or can angiogenesis be induced even in the absence of ischemia? "(page 2320, column 2, last paragraph). They also noted that apart from their preliminary work, all other studies have been performed in the presence of either chronic or acute ischemia and there is a need and more work required to solve the above issue. Since the physiological art is known to be unpredictable, and in the absence of sufficient guidance provided by the instant specification, it would therefore require undue experimentation without a predictable expectation of success for a skilled artisan to make and use the broadly claimed invention.

With regard to claims 25-31 drawn to a method for preventing or reducing the severity of blood vessel damage in a mammal using an effective amount of GM-CSF prior to exposing the mammal to various conditions conducive to damaging blood vessels, the instant specification fails to provide any guidance regarding to the prevention or reduction of the severity of blood vessel damage in a mammal. While the instant specification teaches by exemplification that extensive neovascularization and improved ischemic/normal hindlimb blood pressure ratio were obtained following the

onset of ischemia, as a result of the GM-CSF treatment, these results are not the same or correlated with the contemplated desired therapeutic effects as recited in the preamble of the claims. This is because there is no evidence indicating that **the pre-existing or established blood vessels** exposing to the detrimental conditions would be prevented or subjected to a reduced degree of blood vessel damage as a result of the GM-CSF treatment. It should be noted that any improvement observed in animals treated with GM-CSF relative to untreated control animals is due to **the formation of new blood vessels**. As noted previously, the physiological art is considered to be unpredictive. As such, in the absence of guidance provided by the instant specification and the prior arts at the effective filing date of the present application, it would have required undue experimentation without a predictable expectation of success for one skilled artisan to make and use the instantly claimed invention.

Accordingly, due to the lack of guidance provided by the specification regarding to the aforementioned issues, the unpredictability of the gene therapy art, and the breadth of the claims, it would have required undue experimentation without a predictable degree of success for one skilled in the art to make and use the instant broadly claimed invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3, 4, 14 and 15 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claims 3 and 4, it is unclear what is encompassed by the phrase "increase frequency of endothelial progenitor cells (EPC)". What does it mean? Since the phrase is not defined in the specification, the metes and bounds of the claims can not be clearly determined.

In claims 14 and 15, the phrase "increase EPC bone marrow derived EPC incorporation into foci" is unclear. What do Applicants mean by the term "foci" and what is the relationship between forming foci with the preamble "inducing formation of new blood vessels in a mammal" recited in claim 1 from which both claims 14 and 15 depend upon? Moreover, the phrase "EPC bone marrow derived EPC" is also unclear. Since the phrases are not defined in the specification, the metes and bounds of the claims are not clearly determined.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

Claims 1 and 16-18 are rejected under 35 U.S.C. 102(b) as being anticipated by Pu et al. (Circulation 88:208-215,1993).

The claims are drawn to a method for inducing formation of new blood vessels in a mammal, wherein the method comprises administration to the mammal an effective amount of a vascularization modulating agent sufficient to form the new blood vessels in the mammal; the same wherein the mammal has ischemic tissue or wherein the ischemic tissue is associated with an ischemic vascular disease or wherein the ischemic tissue comprises tissue from a limb, graft or organ.

Pu et al. disclosed enhanced revascularization of the ischemic limb in a rabbit model of persistent hindlimb ischemia by daily intramuscular injections of endothelial cell growth factor for 10 days following the postoperative period. Enhanced neovascularization quantified by angiograms and a better perfusion in the ischemic limb measured by the calf blood pressure ratio were obtained in the treated animals (See abstract). Therefore, the reference clearly anticipates the instant claimed invention.

Claims 1, 2 and 16-20 rejected under 35 U.S.C. 102(b) as being anticipated by Franco (U.S. Patent 4,296,100) or Kawakami et al. (Brain Res. 697:104-111, 1995).

The claims are drawn to a method for inducing formation of new blood vessels in a mammal, wherein the method comprises administration to the mammal an effective amount of a vascularization modulating agent, preferably selected from a group recited in claim 2, sufficient to form the new blood vessels in the mammal; the same wherein

the mammal has ischemic tissue, preferably the ischemic tissue is associated with an ischemic vascular disease or wherein the ischemic tissue comprises tissue from a limb, graft or organ or wherein the tissue is associated with the circulatory system or the central nervous system or wherein the tissue is heart or brain tissue.

Franco teaches a method of treating an area in the heart of a patient subjected to ischemic heart disease to maintain viability in that area for a sustained time period to salvage said area, said method comprising applying an effective dose of FGF to the heart, and wherein the blood flow in said area is increased over that which would occur in the area without treatment with FGF (See examples 1 and 2, and the claims).

Kawakami et al. teach that in an experimental rat model of intracerebral hemorrhage, a local injection of b-FGF into the evacuated cavity resulting from early removal of a mass lesion yielded a protective effect against neuronal damage in CA1 pyramidal cells (probably from ischemia due to the transient mass lesion in the caudate nucleus) and an increase of angiogenesis in the evacuated cavity wall (See abstract, and page 108, column 2, fourth paragraph).

Therefore, both references clearly anticipate the instantly claimed invention.

Claims 1 and 16-20 are rejected under 35 U.S.C. 102(b) as being anticipated by Giordano et al. (Nature Med. 2:534-539, 1996).

Using a pig model of stress-induced myocardial ischemia, Giordano et al. teach that intracoronary injection of a recombinant adenovirus expressing human fibroblast growth factor-5 resulted in the transfer and expression of FGF-5, and regional

abnormalities in stress-induced function and blood flow were improved two weeks following the treatment. Giordano et al. further disclose that the effects persisted for 12 weeks and the improved blood flow and function were associated with evidence of angiogenesis (See abstract). Therefore, Giordano et al. clearly anticipate the instantly claimed invention.

Claims 1 and 16-18 are rejected under 35 U.S.C. 102(b) as being anticipated by Takeshita et al. (Laboratory Investigation 75:487-501, 1996, IDS from 09/265041) or Tsurumi et al. (Circulatioon 94:3281-3290, 1996, IDS from 09/265041) or Isner et al. (The lancet 348:370-374, 1996, IDS from 09/265041).

Takeshita et al. disclose that in a rabbit model of induced hindlimb ischemia, percutaneous delivery to the affected area naked plasmid DNA encoding for $VEGF_{121}$, $VEGF_{165}$ or $VEGF_{189}$ resulted in augmented collateral vessel development and improvement in calf blood pressure ratio (See abstract). Tsurumi et al. also teach that direct intramuscular gene transfer of naked plasmid DNA encoding $VEGF_{165}$ into the affected area resulted in an increase in collateral blood vessel development and tissue perfusion in a rabbit model of induced hindlimb ischemia (See abstract). Isner et al. disclose that arterial transfer of a naked plasmid encoding for $VEGF_{165}$ into a human patient with ischemic limb resulted in an increase in collateral vessels at the knee, mid-tibial, and ankle levels 4 weeks following the treatment (See summary). Therefore, these references clearly anticipate the instantly claimed invention.

Claims 1, 2 and 21-23 are rejected under 35 U.S.C. 102(b) as being anticipated by Bussolino et al. (J. Clin. Invest. 87:986-995, 1991, IDS from 09/265041).

The claims are drawn to a method for inducing formation of new blood vessels in a mammal, wherein the method comprises administration to the mammal an effective amount of a vascularization modulating agent, preferably selected from a group recited in claim 2, sufficient to form the new blood vessels in the mammal; the same wherein the agent is co-administered with at least one angiogenic protein, preferably an endothelial cell mitogen or selected from the group recited in claim 23.

Bussolino et al. disclose that human G-CSF possesses angiogenic activity in the rabbit cornea, although its activity is less than that of bFGF (See abstract and page 988, column 1, first paragraph). Specifically, pellets containing an appropriate dose of G-CSF which were placed into the preformed corneal pockets in rabbits promoted new vessel growth into the implants. Bussolino et al. further teach that when G-CSF and bFGF were placed in adjacent pockets, the combined implantation of G-CSF and bFGF resulted in an angiogenic response exceeding those of the two cytokines alone (page 994, column 1, first paragraph). Thus, Bussolino et al. clearly anticipate the instantly claimed invention.

Claim 48 is rejected under 35 U.S.C. 102(b) as being anticipated by Socinski et al. (The lancet 1:1194-1198, 1988, IDS from 09/265041).

The claim is directed to a method for enhancing endothelial progenitor cell (EPC) mobilization in a mammal, wherein the method comprises administering an effective

amount of at least one hematopoietic factor sufficient to enhance the EPC mobilization in the mammal.

Socinski et al. disclose that purified recombinant human GM-CSF was continuously infused into patients with sarcoma in phase I study (See abstract and page 1195, column 1 under study design section). Since the method in phase I study of Socinski et al. is identical and indistinguishable from the claimed method, the method disclosed by Socinski et al. would inherently enhance the mobilization of EPC in the treated patients. Therefore, Socinski et al. clearly anticipate the instantly claimed invention.

Claim 48 is rejected under 35 U.S.C. 102(e) as being anticipated by Hammond et al. (U.S. Patent 5,880,090, IDS from 09/265041).

Hammond et al. teach that upon administering an agent selected from the group consisting of stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) into a graft recipient, bone marrow-derived CD34+ endothelial progenitor cells are mobilized into the blood stream (increase in the concentration of the progenitor cells) and to enhance the adherence to graft surfaces (See abstract and example 3 in column 9). Therefore, Hammond et al. clearly anticipate the instantly claimed invention.

Applicants should be noted that the article of Grant et al. (Proc. Natl. Acad. Sci. 90:1937-1941, 1993) contains materials related to claims 1 and 2.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 3-6, 11, 12 and 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hammond et al. (U.S. Patent 5,880,090, IDS from 09/265041) in view of Asahara et al. (Science 275:964-967, 1997, IDS from 09/265041).

The claims are drawn to a method for inducing formation of new blood vessels in a mammal, wherein the method comprises administration to the mammal an effective amount of a vascularization modulating agent, preferably selected from a group recited in claim 2, sufficient to form the new blood vessels in the mammal; the same method

wherein the vascularization modulating agent is GM-CSF, and amount of the GM-CSF administered to the mammal is sufficient to increase the concentration of EPC or to increase EPC differentiation in the mammal; the same method wherein the amount of vascularization modulating agent administered to the mammal is sufficient to increase EPC differentiation following tissue ischemia, preferably at least 20% as determined by a standard hindlimb ischemia assay; the same wherein the amount of administered vascularization modulating agent is sufficient to increase EPC bone marrow derived EPC incorporation into foci, preferably at least about 20% as determined by a standard rodent bone marrow transplantation model.

Hammond et al. teach that upon administering an agent selected from the group consisting of stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) into a graft recipient, bone marrow-derived CD34+ endothelial progenitor cells are mobilized into the blood stream (increase in the concentration of the progenitor cells) and to enhance the endothelialization of synthetic vascular grafts (See abstract and example 3 in column 9). Hammond et al. do not teach a method for inducing formation of new blood vessels in a mammal using GM-CSF to mobilize endothelial progenitor cells. However, Hammond et al. noted that Asahara et al. have shown CD34+ endothelial cell populations are capable of differentiating into endothelial-like cells and the circulating CD34+ or Flk-1+ cells may participate in the repair of ischemic tissue (column 3, lines 28-37). In animal models of ischemia (mouse and rabbit models of induced unilateral hindlimb ischemia), Asahara et al. teach that syngeneic or autologous endothelial cell progenitors are

incorporated into capillaries and small arteries in the neovascular zones of the induced ischemic limb (See abstract and page 966).

Accordingly, at the time of the instant invention it would have been obvious to an ordinary skilled artisan to modify the method disclosed by Hammond et al. by administering into a mammal having an ischemia in the limp instead of a recipient of a synthetic vascular graft an agent selected from the group consisting of SCF, GM-CSF and G-CSF to mobilize an effective level of bone marrow-derived endothelial progenitors to home into sites of active angiogenesis to repair the ischemic tissue by forming new blood vessels as taught by Asahara et al. With respect to the limitations recited in claims 4, 6, 12 and 14 regarding to the percentages of increases in the concentrations and differentiation of EPC, these would have been within the scope of skills for an ordinary artisan at the time of the instant invention and also because the assays involved for determining the recited values are standard methods. One of ordinary skilled in the art would have been motivated to carry out the above modification to avoid the tedious and time-consuming isolation and purification of progenitor endothelial cells. Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1, 7-10 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bussolino et al. (J. Clin. Invest. 87:986-995, 1991, IDS from 09/265041) or Pu et al. (Circulation 88:208-215,1993).

The claims are drawn to a method for inducing formation of new blood vessels in a mammal, wherein the method comprises administration to the mammal an effective amount of a vascularization modulating agent sufficient to form the new blood vessels in the mammal; the same wherein the amount of vascularization modulating agent to the mammal is sufficient to increase blood vessel length or further sufficient to increase blood vessel diameter; the same wherein an increase neovascularization by at least 5% as determined by a standard cornea micropocket assay.

Bussolino et al. disclose that human G-CSF possesses angiogenic activity in the rabbit cornea, although its activity is less than that of bFGF (See abstract and page 988, column 1, first paragraph). Specifically, pellets containing an appropriate dose of G-CSF which were placed into the preformed corneal pockets in rabbits promoted new vessel growth into the implants. Bussolino et al. further teach that when G-CSF and bFGF were placed in adjacent pockets, the combined implantation of G-CSF and bFGF resulted in an angiogenic response exceeding those of the two cytokines alone (page 994, column 1, first paragraph). Pu et al. disclosed enhanced revascularization of the ischemic limb in a rabbit model of persistent hindlimb ischemia by daily intramuscular injections of endothelial cell growth factor for 10 days following the postoperative period. Enhanced neovascularization quantified by angiograms and a better perfusion in the ischemic limb measured by the calf blood pressure ratio were obtained in the treated animals (See abstract). Neither Bussolino et al. nor Pu et al. disclose the measurements for new blood vessel length or diameter or report the recited values for an increase in the length of the blood vessel or an increase in neovascularization.

However, it would have been within the scope of skills for an ordinary artisan at the time of the instant invention to do so and because the assays involved for determining the recited values are standard methods. One of ordinary skilled in the art would have been motivated to carry out such measurements to evaluate the potency and determining synergistic effects for various angiogenic proteins or peptides. Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1 and 2 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bussolino et al. (J. Clin. Invest. 87:986-995, 1991, IDS from 09/265041).

The claims are drawn to a method for inducing formation of new blood vessels in a mammal, wherein the method comprises administration to the mammal an effective amount of a vascularization modulating agent sufficient to form the new blood vessels in the mammal; the same wherein the vacularization modulating agent is GM-CSF, M-CSF, b-FGF, SCF, SDF-1, G-CSF, HGF, angiopoietin-1, angiopoietin-2, Flt-3 ligand.

Bussolino et al. disclose that human G-CSF possesses angiogenic activity in the rabbit cornea, although its activity is less than that of bFGF (See abstract and page 988, column 1, first paragraph). Specifically, pellets containing an appropriate dose of G-CSF which were placed into the preformed corneal pockets in rabbits promoted new vessel growth into the implants. Bussolino et al. further teach that when G-CSF and bFGF were placed in adjacent pockets, the combined implantation of G-CSF and bFGF resulted in an angiogenic response exceeding those of the two cytokines alone (page

994, column 1, first paragraph). Although Bussolino et al. do not teach the use of pellets containing the appropriate dose for other recited vascularization modulating agents such as, angiopoietin-1, angiopoietin-2, HGF, it would have been within the scope of skills for an ordinary artisan at the time of the instant invention to do so because the other recited vascularization agents are obvious variants of G-CSF and b-FGF that were used in the work of Bussolino et al. One of ordinary skilled in the art would have been motivated to carry out such modifications to evaluate the potency and determining synergistic effects among various vascularization modulating agents. Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1, 21, 23 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bussolino et al. (J. Clin. Invest. 87:986-995, 1991, IDS from 09/265041) in view of Folkman et al. (J. Biol. Chem. 267, 10931-10934, 1992, IDS from 09/265041) and Rosen et al. (WO 96/39515, IDS from 09/265041).

The claims are drawn to a method for inducing formation of new blood vessels in a mammal, wherein the method comprises administration to the mammal an effective amount of a vascularization modulating agent sufficient to form the new blood vessels in the mammal; the same wherein the agent is co-administered with at least one angiogenic protein, preferably an endothelial cell mitogen or selected from the group recited in claim 23, and more preferably wherein the protein is one of VEGF-B, VEGF-C, VEGF-3.

Bussolino et al. disclose that human G-CSF possesses angiogenic activity in the rabbit cornea, although its activity is less than that of bFGF (See abstract and page 988, column 1, first paragraph). Specifically, pellets containing an appropriate dose of G-CSF which were placed into the preformed corneal pockets in rabbits promoted new vessel growth into the implants. Bussolino et al. further teach that when G-CSF and bFGF were placed in adjacent pockets, the combined implantation of G-CSF and bFGF resulted in an angiogenic response exceeding those of the two cytokines alone (page 994, column 1, first paragraph). Other than bFGF, Bussolino et al. do not teach the use of other endothelial cell mitogens recited in the claims for the co-administration of G-CSF into the rabbit cornea. However, Folkman et al. teach that apart from bFGF other angiogenic polypeptides include aFGF, VEGF, TGF-alpha, TNF-alpha and others (See Table I on page 10932). Additionally, Rosen et al. disclose a novel vascular endothelial growth factor 2 with a potential use for stimulating wound healing and for vascular tissue repair (See abstract).

Accordingly, at the time of the instant invention it would have been obvious to an ordinary skilled artisan to modify the method disclosed by Bussolino et al. by replacing bFGF with any of the angiogenic polypeptides taught by Folkman et al. and Rosen et al. One of ordinary skilled in the art would have been motivated to carry out the above modification to determine the synergistic or additive effects among various angiogenic polypeptides. It should be noted that with respect to other recited endothelial cell mitogens that are not taught by the combined teachings of Bussolino et al., Folkman et

al. and Rosen et al., they are obvious variants. Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 48 and 49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hammond et al. (U.S. Patent 5,880,090, IDS from 09/265041) or Socinski et al. (The lancet 1:1194-1198, 1988, IDS from 09/265041) in view of Folkman et al. (J. Biol. Chem. 267, 10931-10934, 1992, IDS from 09/265041).

The claims is directed to a method for enhancing endothelial progenitor cell (EPC) mobilization in a mammal, wherein the method comprises administering an effective amount of at least one hematopoietic factor sufficient to enhance the EPC mobilization in the mammal; the same method further comprising co-administering to the mammal an effective amount of one or more of granulocyte macrophage-colony stimulating factor, at least one angiogenic protein.

Hammond et al. teach that upon administering an agent selected from the group consisting of stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) into a graft recipient, bone marrow-derived CD34+ endothelial progenitor cells are mobilized into the blood stream (increase in the concentration of the progenitor cells) and to enhance the adherence to graft surfaces (See abstract and example 3 in column 9).

Socinski et al. disclose that purified recombinant human GM-CSF was continuously infused into patients with sarcoma in phase I study (See abstract and page

1195, column 1 under study design section). The method in phase I study of Socinski et al. is identical and indistinguishable from the method in claim 48.

However, neither Hammond et al. nor Socinski et al. teach a further co-administering to the mammal an effective amount of one or more granulocyte macrophage-colony stimulating factor or at least one angiogenic protein. Folkman et al. disclose several polypeptide molecules having angiogenic activity, some of which such as bFGF, aFGF are mitogenic for a wide variety of cell types (See Table 1, page 10932).

It would have been obvious to an ordinary skilled artisan to modify the methods disclosed by Hammond et al. and Socinski et al. by further administering into a graft recipient or the patients another effective amount of GM-CSF or one of several angiogenic polypeptides taught by Folkman et al., respectively. With respect to the study of Hammond et al., one of ordinary skilled in the art would have been motivated to carry out the above modification to further enhance the endothelialization of synthetic vascular grafts because GM-CSF has been shown to mobilize endothelial progenitors to adhere to the graft surfaces and that several angiogenic polypeptides are known to have mitotic activity to increase the proliferation of vascular endothelial cells, for examples VEGF, bFGF and aFGF. With respect to the work of Socinski et al., one of ordinary skilled in the art would have been motivated to co-administer angiogenic proteins such as aFGF and bFGF which are known to exert mitotic activity for a wide variety of cell types, to further expand the circulating hematopoietic progenitor cells in

the patients. Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Conclusions

Claims 25-35 are free of the prior arts. At the time of the instant invention, the prior arts did not teach or fairly suggest methods for preventing or reducing the severity of blood vessel damage in a mammal as claimed.

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Dave Nguyen, may be reached at (703) 305-2024, or SPE, Karen Hauda, at (703) 305-6608.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Patsy Zimmerman, whose telephone number is (703) 305-2758.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1632.

Papers related to this application may be submitted to Group 160 by facsimile transmission. Papers should be faxed to Group 160 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15,1989). The CM1 Fax Center number is or (703) 305-3014 or (703) 308-4242.

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